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ROTHWELL, FIGG, ERNST & MANBECK, P.C.
1425 K STREET, N.W.
SUITE 800
WASHINGTON, DC 20005

EXAMINER	
SHIN, DANA H	

ART UNIT	PAPER NUMBER
1635	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/630,968	ROSSI ET AL.	
	Examiner	Art Unit	
	Dana Shin	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-29* is/are pending in the application.

4a) Of the above claim(s) *24-29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *31July 2003* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *11-03,2-04,8-05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Response to Applicant's Election

Applicant's election without traverse of claims 1-23 in the reply filed on March 21, 2006 is acknowledged. Claims 24-29 are thus withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected groups, there being no allowable generic or linking claim. Accordingly, claims 1-23 are under examination.

Drawings

New corrected drawings for Figures 4-8 in compliance with 37 CFR 1.121(d) are required in this application because it appears that duplicate symbols are used to indicate different siRNA expression cassettes or constructs. Since graphs in Figures 4-8 are presented in non-color drawings, applicants are encouraged to use distinguishable shapes of symbols to indicate each siRNA expression cassette or subject matter shown on the graph in order to demonstrate applicants' data without ambiguity so that the data can be interpreted correctly. Applicant is advised to employ the services of a competent patent draftsperson outside the Office, as the U.S. Patent and Trademark Office no longer prepares new drawings. The corrected drawings are required in reply to the Office action to avoid abandonment of the application. The requirement for corrected drawings will not be held in abeyance.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 18 recites the limitation "the selected cells". There is insufficient antecedent basis for this limitation in the claim because claim 18 refers to the method of claim 17, which does not contain the phrase "selected cells", but "a cell" instead.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 17-23 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the method of an amplification-based method for producing a promoter-containing siRNA expression cassette further comprising transfecting said expression cassette into a mammalian cell *in vitro*, does not reasonably provide enablement for transfecting the mammalian cell of adult origin with the siRNA expression cassette *in vivo* or transfecting the siRNA expression cassette into any mammalian cell *in vivo* for therapeutic purpose. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The breadth of claims 17-23 encompasses both *in vivo* and *in vitro* transfection into mammalian cells. The instant disclosure exemplifies only *in vitro* transfection of siRNA

expression cassettes into mammalian cells but not *in vivo* transfection. Nevertheless, examples 9-13 of the instant specification clearly exemplify siRNA-mediated HIV inhibition and expressly teach that "PCR-amplified expression cassettes expressing anti-tat siRNA were found to potently inhibit HIV infection" (Example 9, paragraph 82). In light of the above, it is clear that the instantly claimed invention embraces a method of transfecting PCR-amplified siRNA expression cassettes into mammalian cells for therapeutic purpose. Although the state of the prior art at the time of the instant invention dated August 1, 2002 (or July 31, 2003) did enable *in vivo* transfection of siRNAs into nematodes (i.e., *C. elegans*. See Fire et al., 1998, Applicant's IDS Citation No. AR) or insect systems (i.e., *Drosophila.* See Kennerdell et al., *Nature Biotechnology*, 17:896-898, 1998) or mouse embryos (i.e., See Wianny et al., *Nature Cell Biology,*1999, Applicant's IDS Citation No. AV), or *in vitro* transfection of siRNAs into mammalian cell lines (See Elbashir et al., 2001, Applicant's IDS Citation No. AE), it did not enable *in vivo* transfection of an siRNA into an adult whole animal or *in vivo* therapy irrespective of the type of siRNA construct (i.e., types of promoter or vector or target gene). The prior art published before August 1, 2002 expressly teaches that the gene inhibition efficacy of *in vivo* administration of siRNAs in adult mammalian organisms or therapeutic usage of siRNAs is not yet explored or examined as stated below:

> "The delivery of siRNAs to the proper sites of therapy remains problematic." (Thomas Tuschl and Arndt Borkhardt, *Molecular Interventions* 2:158-167, June 2002).

> "Finally, this technology (referring to RNA interference) might be used in the future to inhibit the expression of deleterious gene products in selected human cells *in vivo*, with a gene therapy approach." (Bryan R. Cullen, *Nature Immunology* 3:597 – 599, July 2002).

> "If the approaches that we describe can be extended to the adult organism, they will have considerable therapeutic power in inhibiting gene activity in several types of disease." (Wianny et al., *Nature Cell Biology* 2:70-75, February 2000).

In light of the above, it would have been unpredictable whether the claimed invention would have elicited gene silencing in cells, had the expression cassettes been transfected into adult mammalian organisms *in vivo* or used for gene therapy at the time the inventions of claims 17-23 were made, specially since no additional findings regarding the siRNA-mediated gene silencing in late stages of mouse was published and since gene therapy employing siRNAs remained problematic two months before the instant application was filed. Taken together, undue experimentation would have been needed to make and use the claimed invention based on the content of the disclosure (i.e., amount of direction and existence of working examples provided by the inventor) and the state of prior art (i.e., level of one of ordinary skill as well as predictability in the art). Since claims 17-23 embrace non-enabled embodiments, which are not supported by either state of the prior art or direction provided by the inventor in light of the above reasons, it would have been unpredictable whether the claimed invention, embracing *in vivo* transfection into adult mammalian organisms as well as *in vivo* therapy, wherein an siRNA molecule induces gene silencing effects *in vivo,* would have been fully enabled at the time of invention. Due to this unpredictability of the claimed invention, the quantity of experimentation needed to make or use the invention of claims 17-23 based on the content of the disclosure would be undue.

Since the issues described above are not satisfactorily resolved herein, it is concluded, based on the evidence as a whole, that the instant specification fails to teach how to make and use the claimed invention of claims 17-23 without undue experimentation, and that the scope of any enablement provided to one skilled in the art is not commensurate with the scope of protection sought by the claims 17-23.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 22-23 are rejected under 35 U.S.C. 102(a) as being clearly anticipated by Castanotto et al. (*RNA,* 8:1454-1460, 2002).

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed applications, Application Nos. 60/399,718 and 60/408,298, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The above U.S.

Provisional applications do not provide adequate written description for claimed subject matter set forth in claims 22-23. Although the above prior applications provide adequate written description with regard to a PCR-based siRNA expression cassette further comprising the step of transfecting a cell (i.e., 60/399,718 specification pages 3-5, 8-9; 60/408,298 claim 25 and specification paragraph 27), they do not provide any written description with respect to either transfecting cells with two or more different siRNA expression cassettes (instant claim 22) or different siRNA expression cassettes containing different siRNA encoding genes or/and different promoters (instant claim 23). For the reasons stated above, the priority to the prior filed applications for instant claims 22-23 is thus denied, and the filing date of instant application, July 31, 2003, will be the effective filing date for instant claims 22-23.

If applicants believe that the claimed subject matter in claims 22-23 of the instant application is disclosed in the prior-filed applications to which applicants claim the benefit under 35 U. S. C. 119(e), applicants are advised to point out the particulars in response to this Office Action.

Although claims 1-21 are clearly taught by Castanotto et al., they are not subject to the instant rejection because the reference of Castanotto et al. does not antedate the priority date for claims 1-21.

Claims 22-23 are directed to a method of producing a promoter-containing siRNA expression cassette via amplification-based method, further comprising transfecting said cassette into a cell, wherein said cell is transfected with two or more different cassettes containing different siRNA encoding genes.

The reference of Castanotto et al., teaches that the PCR-based amplification method of producing siRNA expression cassettes is useful for the identification of optimal siRNA-target combinations and for multiplexing siRNA expression in mammalian cells (see Abstract, page 1454). It also teaches that the method described above allows several different siRNA gene candidates to be rapidly screened for efficacy (page 1455). Since the method of claims 22-23 is identical to the method taught by Castanotto et al., and the teachings of Castanotto et al., expressly state that different siRNA gene candidates can be co-transfected in cells, all the limitations of the instant claims are met by Castanotto et al.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-5 and 8-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Paddison et al. (*Genes & Development*, 16:948-958, 2002), in view of Tuschl (*Nature Biotechnology,* 20:446-448, 2002), Yu et al. (*PNAS,* Applicants' IDS citation AH, filed on November 19, 2003), Livache et al. (U.S. 5795715, 1998, also Applicant's IDS citation filed on August 15, 2005), and Jones et al. (*Nature,* 344:793-794, 1990).

Claims 1-5 and 8-18 are directed to a PCR-based method for producing a human U6 promoter-containing siRNA expression cassette, wherein the loop sequence connecting sense

and antisense sequences of said siRNA contains about 6 to about 9 nucleotides, said expression cassette is transfected and expressed in mammalian cells, and one or more of oligonucleotide primers are modified by phosphorylation and further comprising screening for a target site of mRNA sensitive to the expressed siRNA.

The reference of Paddison et al., teaches alternative promoter strategies in producing both siRNAs and shRNAs, which is cloned immediately behind the U6 snRNA promoter, thus creating an expression vector for gene silencing. It also teaches that the vector-encoded siRNAs or shRNAs can be synthesized *in vivo* from the U6 snRNA promoter and such expression vectors can stably suppress an endogenous gene in mouse embryo fibroblasts. It further teaches that the preparation of siRNAs (or shRNAs) via expression vectors provides advantages in several aspects such as reduced costs associated with chemical synthesis of siRNAs and a broader application of RNA interference to a larger scale due to convenience and inexpensive method of obtaining siRNAs. It discusses that the siRNA (or shRNA) expression vectors are not only useful in the creation of continuous cell lines in which suppression of a target gene is stably maintained by RNAi, but may also be useful in future for creating transgenic animals as well as *in vivo* and *ex vivo* gene delivery methods for therapeutic approaches based on stable RNAi in humans. With regard to the chemical modifications of siRNAs, the reference of Paddison et al., teaches that RNAs synthesized with RNA polymerase as well as siRNAs in *Drosophila* embryo extracts possess 5' phosphorylated termini and that monophosphoryalted siRNAs are as potent in inducing gene silencing as transcription products with triphosphate termini. Paddison et al., do not teach a method of constructing an siRNA (or shRNA) expression cassette by means of a PCR-based method.

Tuschl reports use of human U6 promoters for expression of siRNAs, which form stem-loop structures having a 9-nucleotide loop sequence and a terminator sequence. Tuschl describes that siRNA expression vectors such as inducible siRNA expression system capable of producing siRNAs, instead of chemically synthesized siRNAs, can be advantageous because a large population of stably-transfected cells could be grown and the construction of siRNA expression vectors is less expensive compared to production of chemically synthesized siRNAs.

The reference of Yu et al., teaches that inhibition by hairpin siRNAs expressed from the U6 promoter-containing siRNA expression vector within mammalian cells is more effective than other methods tested, including the transfection of in vitro-synthesized siRNA duplexes.

The reference of Livache et al., teaches a method of producing a promoter-containing double-stranded RNA expression cassette via a PCR-based method by integrating oligonucleotide primers that are complementary sequences that encompass the sequence of a promoter and the target sequence, wherein the duplex RNA has a defined length (Columns 3 and 6). It discloses that the promoter can be any promoter sequence, and the exemplification of the RNA polymerases of phages T7, T3 or SP6 "does not however represent a limitation because it will appear clearly to a person skilled in the art that any promoter sequence identified as such, and for which the corresponding RNA polymerase is available, can *a priori* be used" (Column 2, lines 55-59).

Jones et al., teach a method of PCR amplifications that accomplishes cloning of PCR product into a vector, wherein both insert and vector are amplified with primers and nested primers which hybridize with a portion of the vector and the target DNA sequence. They demonstrate that the recombinant DNA produced by the PCR amplification method (i.e.,

repeated cycles of denaturing, annealing, and extending of primers and DNA templates) can be transfected into *E. coli* without further use of restriction enzymes or ligase. They discuss that their method, recombinant circle PCR (RCPCR), provides a rapid method for gene fusion and vector reconstruction regardless of enzyme restriction sites, linkers, or adaptors.

It would have been obvious to one of ordinary skill in the art at the time of invention to utilize teachings of Jones et al., in order to rapidly and cost-effectively produce siRNA expression vectors, wherein siRNA sequence is cloned immediately adjacent to the human U6 promoter as taught by Paddison et al., Tuschl, and Yu et al, and to produce an siRNA expression cassette that can produce siRNAs *in vivo* when transfected into mammalian cells.

One of skill in the art would have been motivated to use a method of PCR amplifications to produce an siRNA expression cassette, not only because this method is more rapid and cost-effective than the conventional chemical method of synthesizing siRNAs, as taught by Paddison et al. and Tuschl, but the siRNAs produced by the U6-promoter containing siRNA expression cassettes are more effective in gene silencing than siRNAs chemically synthesized *in vitro*, as taught by Yu et al. Since it has been demonstrated that an expression cassette with the human U6 promoter effectively suppresses gene expression in transfected mammalian cells by teachings of Paddison et al., Tuschl, and Yu et al., one of skill in the art would also have been motivated to replace the human U6 promoter in place of the T7 promoter of Livache et al., in order to express the double-stranded RNA of a defined length in cells. Moreover, since it is well-established in the art that PCR-based amplification can be an efficient, rapid, and inexpensive genetic cloning method as taught by Jones et al., one of skill in the art would have been motivated to practice the PCR-based amplification method to produce a U6 promoter-containing siRNA expression

cassette by using oligonucleotides or primers that hybridize with the gene sequence and the promoter sequence, thus resulting in the practice of the instantly claimed invention with a reasonable expectation of success as Livache et al. has produced a promoter-containing double-stranded RNA expression vector via a PCR amplification method. One of skill in the art would also have been motivated to modify the primer oligonucleotide with phosphorylation because Paddison et al., teach that *in vivo* processed siRNAs contain phosphorylated termini and that the phosphorylated siRNAs are effective in mediating gene silencing. Since it is clearly demonstrated by teachings of Paddison et al., that phosphorylating the siRNA termini is an effective method of gene silencing, one of skill in the art would have been motivated to practice this method with a reasonable expectation of success. Accordingly, the claimed invention, taken as a whole, is *prima facie* obvious over the combined teachings of the prior art.

Claims 6-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tuschl and Livache et al., as applied to claims 1-5 and 8-18 above, and further in view of Jeng et al. (*JBC*, 267:19306-19312, 1990).

Claims 6-7 are drawn to an amplification-based method for producing a promoter-containing siRNA expression cassette, wherein the terminator sequence comprises a sequence of about 4-6 deoxyadenosines (claim 6) or 6 deoxyadenosines (claim 7).

Jeng et al., teach an RNA hairpin structure with an adjacent, downstream deoxyadenosine as well as deoxythymidine-rich regions, one of which contains a continuous sequence of deoxyadenosine residues within which the transcript terminates. They report that the length of the deoxyadenosine residues is critical for termination efficiency and show that at least 5

deoxyadenosine residues are necessary to begin to cease synthesis and 8 or 9 are required for maximal termination for T7 RNA polymerase.

It would have been obvious to one of ordinary skill in the art at the time of invention to modify teachings of Jeng et al., in order to produce an siRNA expression cassette that contains a functionally efficient transcription terminator. One of skill in the art would have been motivated to perform experiments to obtain the most optimal length of deoxyadenosine residues because Jeng et al., teach that the length of the deoxyadenosine residues is critical for efficient termination of transcript. Since Jeng et al., have already reported that the terminator sequence should comprise at least 5 deoxyadenosine residues, one of skill in the art would have been motivated to insert at least 5 deoxyadenosine residues as a terminator sequence in the siRNA expression cassette with a reasonable expectation of success in terminating transcript of the sense and antisense strands of the siRNA. Accordingly, the claimed invention, taken as a whole, is *prima facie* obvious over the combined teachings of the prior art.

Claims 22-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gou et al. (*FEBS Letters,* 548:113-118, published online on July 5, 2003), in view of Paul et al. (*Nature Biotechnology,* Applicant's IDS citation AG, filed on November 19, 2003).

Claims 22-23 are directed to a method of producing a promoter-containing siRNA expression cassette further comprising transfecting said cassette into a cell, wherein said cell is transfected with two or more different cassettes containing different siRNA encoding genes.

The reference of Gou et al., teaches the instantly claimed method. It does not teach that different siRNA expression cassettes containing different siRNA encoding genes can be simultaneously transfected into a cell.

The reference of Paul et al., teaches that it is possible to use several cassettes targeting multiple mRNAs, and in fact, targeting two different genes, human splicing factor and HIV-1 reverse transcriptase coding region, have already been shown to be an effective (p507).

It would have been obvious to one of ordinary skill in the art at the time of invention to combine teachings of Gou et al. and Paul et al., in order to practice the method of producing siRNA expression cassettes containing different siRNA encoding genes and simultaneously transfect the cassettes into cells. One of skill in the art would have been motivated to do so because targeting different genes simultaneously by siRNA expression cassettes produced in a fast and less-expensive manner would allow a person of skill in the art to suppress multiple genes at a time to study antagonistic or synergistic mechanisms of different endogenous genes in a biological system. Furthermore, since Paul et al., have already reported that the targeting different genes simultaneously by using U6-driven siRNA expression cassettes has effectively silenced the two very different genes in cells, one of skill in the art would have been motivated to practice the combined teachings of Gou et al. and Paul et al., with a reasonable expectation of success in suppressing expression of different target genes via a single transfection assay. Accordingly, the claimed invention, taken as a whole, is *prima facie* obvious over the combined teachings of the prior art.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Dana Shin whose telephone number is 571-272-8008. The examiner can normally be reached on Monday through Friday, from 8am-4:30pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on 571-272-4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Dana Shin
Examiner
Art Unit 1635

D. Shin
5-17-2006

JAMES SCHULTZ, PH.D.
PRIMARY EXAMINER